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(71) Applicants: CT-HOLDING S.A. [CH/CH]; 30, route des Gouttes-d'Or, CH-2000 Neuchâtel (CH). CT-HOLDING S.A. [CH/US]; 2700 Bay Avenue, Sunset Island, Number 2, Miami Beach, FL 33140 (US).

(72) Inventors: SAUER, Gerhard; Muhlweg 12-4, D-69118 Heidelberg (DE). AMTMANN, Eberhard; Mozart Strasse 29, D-69121 Heidelberg (DE).

(74) Agents: EVANS, Barry et al.; Curtis, Morris & Safford, P.C., 530 Fifth Avenue, New York, NY 10036 (US).

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(54) Title: ANTIVIRAL AND ANTITUMOR PHARMACEUTICAL COMPOSITIONS

(57) Abstract

This invention relates to compositions comprising a pharmaceutically effective amount of a xanthate compound and an adjuvant in a lipid-based or steroid-based carrier which are useful for the treatment of viruses and tumors. In particular, the compositions of the invention are effective for the treatment of HSV.

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ANTIVIRAL AND ANTITUMOR PHARMACEUTICAL COMPOSITIONS

FIELD OF THE INVENTION

The invention relates broadly to pharmaceutical compositions for the treatment of viral diseases and tumors. More specifically, the invention relates to compositions containing xanthate compounds and activity enhancing adjuvants in lipid- or steroid-based carriers.

BACKGROUND OF THE INVENTION

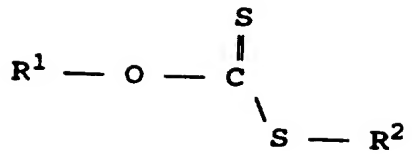
Viral diseases and tumorigenic diseases are a major cause of mortality in man and animals. Lack of success in prior treatments is due primarily to the fact that both diseases are closely associated with the affected cells, e.g. replication of viruses is driven by the host cell biomechanics and tumor growth develops from preexisting tissue.

Of particular medical concern is the herpes simplex virus (HSV). HSV is a serious and widespread health problem of epidemic proportion, due in large part to the proclivity of the virus to establish a latent infection and thereafter to produce spontaneous recurrent disease. Dr. Jonas Salk, in Prospects for the Control of AIDS by Immunizing Seropositive Individuals, Nature (London) Vol. 327 (1987) pgs. 473-476, estimated that up to 50% of teenagers, from a range of socioeconomic backgrounds and demographic locations, will be HSV seropositive by the age of 16. An HSV seropositive response indicates a past history of either HSV type 1 (HSV-1) or type 2 (HSV-2) infection.

An important obstacle to the development of antiviral and antitumor treatments is the development of a suitable delivery system that can target therapeutic agents in effective concentrations to sites of virus replication or tumor growth.

U.S. Patent No. 4,602,037 issued July 22, 1986 to Scherm et al. describes the antiviral and antitumor properties of xanthates. The disclosure of U.S. Patent No. 4,602,037 is hereby incorporated by reference.

The xanthates described in U.S. Patent No. 4,602,037 fall within the scope of formula I:



(I)

wherein R^1 represents norbornyl, tricyclodecyl (including adamantyl), benzyl, straight or branched C_3-C_{20}-alkyl, C_3-C_{20}-cycloalkyl, furyl, pyridyl, or quinuclidinyl or the aforesaid straight or branched C_3-C_{20}-alkyl optionally substituted by hydroxy, C_1-C_4-alkoxy, or by halogen, or the aforesaid C_3-C_{20}-cycloalkyl optionally substituted by hydroxy, C_1-C_4-alkoxy, C_1-C_4-alkyl, or halogen; and wherein R^2 represents a monovalent or multivalent metal atom, straight or branched C_1-C_6-alkyl, which may optionally be substituted by hydroxy, C_1-C_4-alkoxy, amino, C_1-C_4-alkylamino, $(C_1$-C_4-alkyl$)_2$-amino, $(C_1$-C_4-alkyl$)_3$-ammonium, or halogen, or 2,3-dihydroxypropyl or ω-hydroxy-(C_1-C_4-alkoxy)-methyl.

Sodium or potassium benzylxanthate, cyclohexylxanthate, 1-adamantylxanthate, 8(9)-tricyclo[5.2.1.$0^{2.6}$]-decylxanthate, 2-endo or exo-bicyclo[2.2.$1^{1.4}$]-heptylxanthate, cyclododecylxanthate, n-dodecylxanthate, or 4-isobornyl-cyclohexylxanthate are compounds which have been found to be particularly effective.

Numerous xanthates of such structure have been tested for their antiviral characteristics. Sauer et al., "DNA and RNA Virus Species are Inhibited by Xanthates, A Class of Antiviral Compounds With Unique Properties," Proc. Natl. Acad. Sci., Vol. 81 (June 1984) pp. 3263-3267 discuss the testing of the following compounds:

D416: Cyclohexyl-oxy-dithioformic-acid sodium salt

D435: Cyclododecyl-oxy-dithioformic-acid potassium salt

D436: Dodecyl-oxy-dithioformic-acid potassium salt

D442: Toluoyl-oxy-dithioformic-acid sodium salt
D607: Cyclohexyl-oxy-dithioformic-acid-methyl ester
D609: Tricyclo[5.2.1.$0^{2,6}$]-decyl-oxy-dithioformic-acid potassium salt
D611(endo): 2-endo-bicyclo[2.2.1]-heptyl-dithioformic-acid potassium salt
D611(exo): 2-exo-bicyclo[2.2.1]-heptyl-dithioformic-acid potassium salt
D614: Cyclohexyl-oxy-dithioformic-acid dimethylglycl-ester

All of these compounds exhibited antiviral activity. In particular, D435, D609, and D611 were found to be very efficient virus inhibitors.

It is also known that certain compounds can be used as adjuvants to increase the effectiveness of therapeutic compounds. Adjuvants, by themselves, do not exhibit therapeutic qualities, but when combined with therapeutic compounds enhance their effectiveness.

U.S. Patent No. 4,851,435 issued July 25, 1989 to Sauer et al. describes using xanthates, as described in U.S. Patent No. 4,602,037, in conjunction with certain adjuvants. The disclosure of U.S. Patent No. 4,851,435 is hereby incorporated by reference.

The adjuvants described in U.S. Patent No. 4,851,435 are ionic compounds having both lipophilic and hydrophilic groups. The compound is desirably one wherein the lipophilic group is a straight or branched aliphatic group with 6 to 18 carbon atoms and the hydrophilic group comprises 1 or 2 carboxyl and/or 1 or 2 sulphate, sulphonate, or phosphate groups. Advantageously, the adjuvant compound is an aliphatic mono or dicarboxylic acid, or fluorinated derivative thereof, or an aliphatic mono or disulphate, mono or disulphonate, or mono or diphosphate, and has 6 to 18 carbon atoms, or, such a compound having 1 or 2 ether

and/or amide groups. Pharmaceutically acceptable salts of all the above compounds may be used.

Preferably the adjuvant compounds are aliphatic monocarboxylic acids with 9 to 13 carbon atoms or fluorinated derivatives thereof, or fatty alcohol sulphates, phosphates, ether phosphates, ether sulphates, alkane sulphonates, olefinic sulphonates, sulphocarboxylic acid esters or glyceride sulphates having 8-18 carbon atoms. Naturally occurring fatty acids or fatty alcohol sulphates with 8 to 18 carbon atoms are also effective. The most advantageous adjuvant compounds are the sodium and potassium salts of decanoic acid, undecanoic acid, dodecanoic acid, deoxycholic acid, dodecyl sulfate, or dodecylphosphonic acid, or pharmaceutically acceptable salts thereof.

A number of the above-discussed adjuvants have been tested for their antiviral enhancing characteristics. Music et al., "Mechanistic Aspects of the Synergistic Antiviral Effect of Xanthates and Monocarbonic Acids," Biochemical Pharmacology, Vol. 38, No. 12 (1989) pp. 1941-1945 discusses the testing of the adjuvants with the xanthate D609 (8(9)-tricyclo[5.2.1.$0^{2,6}$]-decyl xanthate). Fatty acids of eleven to fourteen carbon atoms (undecanoic acid, dodecanoic acid and myristic acid) were found to be effective adjuvants while shorter (6 carbon) and larger (18 carbon) monocarboxylic acids were shown to lack activity enhancing properties.

The xanthate and xanthate/adjuvant compositions have been found to have effective antiviral and/or antitumor activity if the requisite concentration is at least 2.5 wt% xanthate in a topical ointment and at least 10 mg/ml xanthate in a solution for intravenous or subcutaneous injection.

When using xanthate/adjuvant compositions for topical application, it has been found that experimental animals can only tolerate a concentration of about 1 wt%

xanthate in ointment. A concentration of 3 wt% xanthate causes skin irritation and a concentration of 5 wt% xanthate causes necrotic destruction of tissue. The 1% concentration is much too low to achieve the desired therapeutic effect.

When a xanthate/adjuvant composition was prepared as a solution for intravenous or subcutaneous injection, concentrations above 1 mg/ml could not be tolerated by experimental mice without severe destruction of tissue. This concentration also is well below the levels necessary to achieve the desired therapeutic effect.

The challenge in producing a therapeutically effective composition is to develop an effective carrier to reduce the toxicity of the xanthates such that the antiviral and antitumoral xanthates can be delivered in effective concentrations to sites of virus replication or tumor growth.

OBJECTS OF THE INVENTION

It is thus a primary object of the invention to provide a composition capable of delivering an effective amount of a xanthate compound plus adjuvant to sites of virus replication or tumor growth for combating said viruses or tumors.

It is a further object of this invention to provide a carrier for a xanthate plus adjuvant composition which reduces the toxicity of the active components.

It is still a further object of this invention to provide a xanthate compound plus adjuvant composition in a carrier which can be used in topical applications or in intravenous or subcutaneous injections.

SUMMARY OF THE INVENTION

These and other objects of the invention are achieved in compositions comprising a pharmaceutically effective amount of a xanthate compound and an adjuvant in a lipid-based or steroid-based carrier. These

compositions are useful for the treatment of viruses and tumors. In particular, the compositions of the invention are effective for the treatment of HSV.

In preferred embodiments, the invention includes, as the active components, a xanthate which has antiviral or antitumoral activities, such as those described in U.S. Patent No. 4,602,037, and, an ionic adjuvant containing both a lipophilic and hydrophilic group which has been shown to enhance the activity of the xanthate, such as those described in U.S. Patent No. 4,851,435, and, as a carrier, cholesterol.

A particularly preferred composition includes the active components (a) a sodium or potassium salt of 8(9)-tricyclo[5.2.1.$0^{2,6}$]-decylxanthate (D609), and (b) a sodium or potassium salt of lauric acid (KC12), also known as dodecanoic acid, and in a liposome comprised of cholesterol.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph showing the irritating effects of D609-containing ointments.

FIG. 2 is a graph showing the irritating effect of D609-containing ointments, in cholesterol and in free-form, respectively, when topically administered to mice.

FIG. 3 is a graph showing the irritating effect of D609-containing solutions, where the D609/KC12 is in free-form and in cholesterol, respectively, when subcutaneously injected in mice.

FIG. 4 is a graph of the dose-response curves of free-form D609/KC12, and D609/KC12 in cholesterol, respectively, when used to treat monkey kidney cells (Rita) infected with herpes simplex virus type 1 (HSV-1).

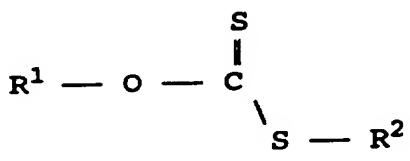
FIG. 5 is a graph showing the response of tumors in mice to D609/KC12 in cholesterol over a two week period.

DETAILED DESCRIPTION OF THE INVENTION

The objects of the invention are achieved by compositions comprising an effective amount of a xanthate

compound and an adjuvant in a lipid- or steroid-based carrier.

Broadly, the compositions include a xanthate compound of formula I:



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wherein R^1 represents norbornyl, tricyclodecyl (including adamantyl), benzyl, straight or branched C_3-C_{20}-alkyl, C_3-C_{20}-cycloalkyl, furyl, pyridyl, or quinuclidinyl or the aforesaid straight or branched C_3-C_{20}-alkyl optionally substituted by hydroxy, C_1-C_4-alkoxy, or by halogen, or the aforesaid C_3-C_{20}-cycloalkyl optionally substituted by hydroxy, C_1-C_4-alkoxy, C_1-C_4-alkyl, or halogen; and wherein R^2 represents a monovalent or multivalent metal atom, straight or branched C_1-C_6-alkyl, which may optionally be substituted by hydroxy, C_1-C_4-alkoxy, amino, C_1-C_4-alkylamino, $(C_1$-C_4-alkyl$)_2$-amino, $(C_1$-C_4-alkyl$)_3$-ammonium, or halogen, or 2,3-dihydroxypropyl or ω-hydroxy-(C_1-C_4-alkoxy)-methyl;

an adjuvant compound, generally an ionic compound having both lipophilic and hydrophilic groups, wherein the lipophilic group is a straight or branched aliphatic mono or dicarboxylic acid, or fluorinated derivative thereof, or an aliphatic mono or disulphate, mono or disulphonate, or mono or diphosphate, having 6 to 18 carbon atoms, or such a compound having 1 or 2 ether and/or amide groups, and wherein the hydrophilic group comprises 1 or 2 carboxyl and/or 1 or 2 sulphate, sulphonate, or phosphate groups, or pharmaceutically acceptable salts thereof; and

a lipid-based or steroid-based carrier.

The preferred embodiment of the invention is the sodium or potassium salt of 8(9)-tricyclo[5.2.1.$0^{2,6}$]-decylxanthate (D609) mixed with the sodium or potassium

salt of lauric acid, also known as dodecanoic acid (KC12) in cholesterol.

The carriers of the invention are lipid-based or steroid-based carriers. The lipid-based carrier are amphipathic lipids including phospholipids (e.g. lecithin, phosphatidylcholine, phosphatidylserine, phosphatidylinositol), glycolipids (e.g. ganglioside), sphingolipids (e.g. sphingomyelin). The steroid-based carriers include stearylamine, chondrillasterol, α,β,γ sitosterol, cholesterol and its salts, and cholesterol derviatives such as cholestanol and cholanic acid. The carrier must be pharmaceutically acceptable and compatible with the active components, xanthate and adjuvant.

The active components may be suspended within the carrier, may form micelles therewith, may be micro-emulsified within the carrier or may be encapsulated within a liposome structure.

Liposomes are generally spherical bilayer lipid structures having aqueous interiors. They are prepared by suspending a polar lipid film, such as a phospholipid, in an aqueous solution. They have basically the same structure as do cell membranes and therefore have many properties similar to those cell membranes. Liposomes are easy to manipulate mechanically, their compositions can be varied, and, they have the ability to encapsulate or complex a wide variety of hydrophilic or lipophilic biologically active compounds. Liposomes can be formed from a variety of substances, such as phospholipids, glycolipids, sphingolipids, and steroids. Numerous methods exist to make liposomes. These include sonication, ultrasonication, injection, centrifugation, entrapment by freezing, and dehydration/rehydration.

Of particular importance as a carrier is the steroid cholesterol. Cholesterol is the major constituent of animal tissue, and although cholesterol is almost entirely hydrocarbon in composition, it is

amphipathic because it contains a hydroxyl group that interacts with water. This characteristic makes cholesterol a particularly effective carrier for therapeutic compounds. The active components may be micro-emulsified in the cholesterol, forming a micelle therewith or be microencapsulated in a liposome thereof.

Surprisingly, it has now been found that when the xanthate/adjuvant mixtures are incorporated into cholesterol, concentrations of up to 12.5 wt% D609 in topical ointments and up to 50 mg/ml D609 in solutions for injection were tolerated by experimental mice. Surprisingly, it was also found that the incorporation of the active components in cholesterol had no adverse effect upon the antiviral activity of the xanthate/adjuvant mixture.

Preparation of Therapeutic Compositions

A therapeutic composition can be prepared by mixing approximately equal amounts of D609 and KC12 with cholesterol. The preferred ratio of cholesterol to D609/KC12 mixture is about 1:1. Sterile, pyrogen-free water is added to the D609/KC12/cholesterol mixture to form a suspension. The suspension is then sonified and centrifuged to form an emulsion. The term emulsion is used herein but may be understood to cover emulsions, micelles, liposomes and other complexations of the active compounds in the cholesterol. The emulsion is immediately frozen and lyophilized.

It has been found that solution emulsions of D609/adjuvant mixtures in cholesterol, in a ratio of one part xanthate, one part adjuvant and two parts cholesterol, can be prepared for intravenous or subcutaneous injection by addition of a buffer solution (aqueous NaCl). Such solution emulsions may contain concentrations of D609 up to 50 mg/ml. Solution emulsions containing higher concentrations were too viscous to be effectively used for injections.

Ointments for topical treatment can be prepared with liquid paraffin and vaseline. These contain the active components in a ratio of one part xanthate, one part adjuvant and two parts cholesterol. Concentrations of D609 up to 12.5 wt% in the ointment can be obtained. The upper limit of concentration is a physical limitation. The ointment becomes too viscous at higher concentrations to be effectively used for topical application.

It has been found that an effective xanthate to adjuvant ratio is one to one. However, the xanthate to adjuvant ratio can vary from one part xanthate per ten parts adjuvant to ten parts xanthate per one part adjuvant. Such mixtures, when incorporated in the carriers of the invention, will exhibit effective antiviral and/or antitumor activity.

It has also been found that the ratio of xanthate/adjuvant mixture to cholesterol may be broadly from one part xanthate/adjuvant mixture to 0.25 part cholesterol to one part xanthate/adjuvant mixture to 4 parts cholesterol.

When administered in a cholesterol carrier, the xanthate/adjuvant mixture retains its therapeutic effectiveness, and is surprisingly and significantly less toxic. Most surprising was the discovery that the xanthate alone cannot be effectively incorporated into cholesterol without the adjuvant.

The following examples are presented to illustrate and provide a better understanding of the invention.

EXAMPLE 1

Incorporation of D609 in Cholesterol
With and Without Lauric Acid

D609 (without KC12) in cholesterol was prepared by mixing 400 milligrams of D609 and 400 milligrams of cholesterol suspended in a 50 ml plastic vial in 40 ml distilled water. D609 (with KC12) in cholesterol was

prepared by mixing 400 milligrams of D609, 400 milligrams of KC12, and 800 milligrams of cholesterol suspended in a 50 ml plastic vial in 40 ml distilled water. Both suspensions were placed in an external ice bath and sonified with a Branson Sonifier B15 at a maximum energy output with 40% duty cycle for 15 minutes to form an emulsion.

Free D609 was separated from the suspensions by ultrafiltration (Amicon micro ultra-filtration vials, exclusion size 30kd, Sorvall centrifuge SS20 rotor at 5000 rpm for 15 minutes) after 1:10 dilution with phosphate buffered isotonic salt solution (pH 7.0). Five µl of each sample were applied to a Silia Gel 60 plate and the plate was run in acetonitrile. Aliquots (1-7 µl) of a solution containing 1 mg/ml D609 served as position markers and for quantitative determination.

D609 spots were visualized under ultraviolet light (300nm). The absorption of each spot was quantitated with the aid of a computerized video system.

The resulting analysis indicated that in the presence of KC12, 87% of D609 was incorporated in the cholesterol. However, in the absence of KC12, only 1% of D609 was incorporated.

Additional experiments were run to confirm that the presence of KC12 was necessary for effective incorporation of D609. The confirmatory results are indicated in Table 1.

TABLE 1

D609 conc. (mg/ml)	KC12 conc. (mg/ml)	Cholesterol conc. (mg/ml)	Amount of incorporated D609 (%)
50	0	200	0.8
50	20	200	35
50	50	200	81
20	50	200	60
10	50	200	45
50	0	500	6.8
45	5	500	76.3
35	15	500	83.5
25	25	500	83.3
15	35	500	76.6
5	45	500	71.0

EXAMPLE 2

Preparation of Compositions Containing D609/KC12 in Cholesterol For Therapeutic Test Purposes

The composition was prepared by mixing 2 grams of D609 and 2 grams of KC12 with 4 grams of cholesterol and sterile, pyrogen-free water was added to make up a final volume of 40 ml of suspension. The suspension was placed in an external ice bath and sonified with a Branson Sonifier B15 at a maximum energy output with 40% duty cycle for 15 minutes to form an emulsion. The emulsion was then centrifuged at about 3000 Gs for approximately 10 minutes. After centrifugation, the volume of the supernatant was adjusted to about 40 ml by the addition of distilled water. The emulsion was immediately frozen and lyophilized.

Solution emulsions for intravenous or subcutaneous injection were prepared by the addition of a saline buffer solution (0.9% NaCl) to the D609 emulsion. Solution emulsions with up to 50 mg/ml of D609 can be prepared. Ointments for topical treatments were prepared

by mixing the solution emulsions with liquid paraffin and vaseline. Concentrations of D609 of up to 12.5 wt% were obtained.

EXAMPLE 3

Assessment of the Irritating Effects of D609-Containing Ointments

In order to assess the irritating potential of D609-containing ointments, ointments containing various amounts of D609 in vaseline were administered to the each of the shoulder regions of four female mice (strain NMRI Nu/Nu, 8 weeks old). The degree of irritation was scored beginning 16 hours after treatment according to the following stages: no effect (0), slight redness (0.5), redness (1.0), inflammation (1.5), and visible tissue damage (2.0).

When examined 16 hours after treatment, the ointment containing 1.56 wt% free D609 had no effect and was given an irritation score of 0. The ointment with 3.13 wt% free D609 caused slight reddening in 2 sites, reddening in 3 sites, tissue destruction in 1 site, and had no effect in 1 site, and was given an irritation score of 6. The ointment with 6.25 wt% free D609 caused redness in 2 sites, tissue damage in 5 sites, and no effect in 1 site, and was given an irritation score of 12. The ointment with 12.5 wt% free D609 caused reddening in 1 site, inflammation in 1 site, and tissue damage in the remaining 6 sites, and was given an irritation score of 14.5.

The results of this study are graphically represented in FIG 1.

EXAMPLE 4

Assessment of the Irritating Effect of D609/KC12-Containing Ointments

D609/KC12 in Free Form

In order to assess the irritating potential of D609/KC12-containing ointments, ointments containing equal amounts of D609 and KC12 in free-form were

administered to the flanks of eight female, 10 week old, nude mice twice daily. Four animals were treated on each flank with D609/KC12 ointment. The degree of irritation was scored beginning 4 hours after the initiation of treatment according to the following stages: no effect (0), slight redness (0.5), redness (1.0), inflammation (1.5), and visible tissue damage (2.0).

When examined 4 hours after treatment, the ointment containing 1.25 wt% free D609/KC12 caused reddening in 1 out of 8 application sites, and was given an irritation score of 1. The ointment with 2.5 wt% free D609/KC12 caused reddening in 3 sites, and was given an irritation score of 3. The ointment with 5 wt% free D609/KC12 caused redness in two sites and tissue damage in the remaining six sites, and was given an irritation score of 14.

D609/KC12 in Free Form

The same protocol was followed except that the D609/KC12 mixture was incorporated in cholesterol. When examined 4 hours after treatment, the ointment with D609/KC12 in cholesterol was very well tolerated. The concentrations of D609 had to be increased to 12.5 wt% in order to cause slight reddening in two sites. This test was given an irritation score of 1.

The reddening of the skin caused by the D609/KC12 in cholesterol was found to be transient and disappeared within 16 hours after termination of the treatment. The tissue damage, in contrast, which was caused by free D609/KC12 (5 wt%), failed to disappear within this period of time.

The results of this study are graphically represent in FIG 2.

EXAMPLE 5

Assessment of the Irritating Potential of D609-Containing Solutions After Subcutaneous Injection

In order to assess the irritating potential of D609-containing solutions after subcutaneous injection,

0.1 ml of solutions containing varying amounts of free D609, D609/KC12 mixtures and D609/KC12 mixtures in cholesterol were subcutaneously injected into four female mice (strain NMRI Nu/Nu, 8 weeks old) in both flanks. The degree of irritation was scored 16 hours after treatment according to the following stages: no effect (0), slight redness (0.5), redness (1.0), inflammation (1.5), and visible tissue damage (2.0).

The summarized scores of each treatment are indicated in Table 2 and FIG. 3.

TABLE 2

D609 conc. (mg/ml)	Irritation score of free D609	Irritation score of free D609/KC12	Irritation score of D609/KC12 in cholesterol
0.63	0.5 (0,0,0,.5)	0.0 (0,0,0,0)	no data
1.25	2.5 (0,.5,.5,1.5)	2.0 (0,0,.5,1.5)	no data
2.5	5.0 (1.5,1.5,1.5,2)	4.5 (0,.5,1.5,1.5)	no data
5.0	6.5 (1.5,1.5,1.5,2)	6.5 (1,1.5,2,2)	0.0
10.0	8.0 (2,2,2,2)	8.0 (2,2,2,2)	no data
25.0	no data	no data	0.5 (0,0,0,.5)
50.0	no data	no data	0.0 (0,0,0,0)

EXAMPLE 6

Antiviral and Antitumor Activity of D609 Incorporated in Cholesterol

A. Antiviral Activity of the D609/KC12 Mixtures in Free Form and in Cholesterol

Monkey kidney cells (Rita) were seeded in Linbro plates (4 x 10^6 each). After one day, the cells were infected with 100 pfu of herpes simplex virus type 1 (HSV-1) per well. After about one hour absorption, fresh

tissue culture medium (Basal medium Eagle; 10% fetal calf serum, pH 7.4) containing concentrations of either free D609/KC12 (10 mg/ml in acetone) or D609/KC12 incorporated in cholesterol (10 mg/ml in 0.9% NaCl buffer solution) was added (2 wells each). One day later, the tissue culture medium was omitted, the cells were fixed with 3% formaldehyde and stained with 0.5% cristal violett. Plaques were then counted and the mean values calculated.

FIG. 4 shows the comparison of the dose-response curve for free D609/KC12 and D609/KC12 incorporated in cholesterol, respectively. The study demonstrates that there was no loss of antiviral activity by incorporation of the active components in cholesterol.

B. Antitumor Activity of D609/KC12 Mixtures in Cholesterol

Human colorectal carcinoma cells (5 millions in 0.1 ml isotonic salt solution) were injected subcutaneously into both flanks of athymic nude mice (NMRI, 8 weeks old). After 10 days, tumors appeared and the mice were treated subcutaneously at the site of the tumors with a 0.2 ml of either a control solution of isotonic salt solution (placebo) or a test solution containing D609/KC12 incorporated in cholesterol having a concentration of 10mg/ml of D609.

FIG. 5 and Table 3 show the treatment response to D609/KC12 in cholesterol. The mean values of relative tumor sizes (tumor size at the beginning of treatment = 100%) are given over a two week period. The study demonstrates that the xanthate/adjuvant mixture in cholesterol had excellent antitumor activity.

TABLE 3

Days After Beginning Treatment	Placebo Mean Tumor Size (%)	D609 in Cholesterol Mean Tumor Size (%)
0	100	100
2	185	40
3	226	53
7	584	48
10	887	55
14	1031	21

EXAMPLE 7

Preparation of Compositions Containing D609/KC12 in the Lipid-Based Carrier Lecithin For Therapeutic Test Purposes

The composition is prepared in a manner similar to that of Example 2 by mixing 2 grams of D609 and 2 grams of KC12 with 4 grams of lecithin. Sterile, pyrogen-free water is added to make up a final volume of 40 ml of suspension. The suspension is placed in an external ice bath and sonified. The results achieved with this composition are similar to the results achieved with the composition of Example 2, with respect to skin irritation levels (Examples 4 and 5), and with respect to antiviral and antitumor activity (Example 6).

EXAMPLE 8

Preparation of Compositions Containing D609/KC12 in the Lipid-Based Carrier Phosphatidylcholine For Therapeutic Test Purposes

The composition is prepared in a manner similar to that of Example 2 by mixing 2 grams of D609 and 2 grams of KC12 with 4 grams of phosphatidylcholine. Sterile, pyrogen-free water is added to make up a final volume of 40 ml of suspension. The suspension is placed in an external ice bath and sonified. The results

achieved with this composition are similar to the results achieved with the composition of Example 2, with respect to skin irritation levels (Examples 4 and 5), and with respect to antiviral and antitumor activity (Example 6).

EXAMPLE 9

Preparation of Compositions Containing D609/KC12 in the Lipid-Based Carrier Phosphatidylserine For Therapeutic Test Purposes

The composition is prepared in a manner similar to that of Example 2 by mixing 2 grams of D609 and 2 grams of KC12 with 4 grams of phosphatidylserine. Sterile, pyrogen-free water is added to make up a final volume of 40 ml of suspension. The suspension is placed in an external ice bath and sonified. The results achieved with this composition are similar to the results achieved with the composition of Example 2, with respect to skin irritation levels (Examples 4 and 5), and with respect to antiviral and antitumor activity (Example 6).

EXAMPLE 10

Preparation of Compositions Containing D609/KC12 in the Lipid-Based Carrier Phosphatidylinsitol For Therapeutic Test Purposes

The composition is prepared in a manner similar to that of Example 2 by mixing 2 grams of D609 and 2 grams of KC12 with 4 grams of phosphatidylinositol. Sterile, pyrogen-free water is added to make up a final volume of 40 ml of suspension. The suspension is placed in an external ice bath and sonified. The results achieved with this composition are similar to the results achieved with the composition of Example 2, with respect to skin irritation levels (Examples 4 and 5), and with respect to antiviral and antitumor activity (Example 6).

EXAMPLE 11

Preparation of Compositions Containing D609/KC12 in the Lipid-Based Carrier Ganglioside For Therapeutic Test Purposes

The composition is prepared in a manner similar to that of Example 2 by mixing 2 grams of D609 and 2 grams of KC12 with 4 grams of ganglioside. Sterile, pyrogen-free water is added to make up a final volume of 40 ml of suspension. The suspension is placed in an external ice bath and sonified. The results achieved with this composition are similar to the results achieved with the composition of Example 2, with respect to skin irritation levels (Examples 4 and 5), and with respect to antiviral and antitumor activity (Example 6).

EXAMPLE 12

Preparation of Compositions Containing D609/KC12 in the Lipid-Based Carrier Sphingomyelin For Therapeutic Test Purposes

The composition is prepared in a manner similar to that of Example 2 by mixing 2 grams of D609 and 2 grams of KC12 with 4 grams of sphingomyelin. Sterile, pyrogen-free water is added to make up a final volume of 40 ml of suspension. The suspension is placed in an external ice bath and sonified. The results achieved with this composition are similar to the results achieved with the composition of Example 2, with respect to skin irritation levels (Examples 4 and 5), and with respect to antiviral and antitumor activity (Example 6).

EXAMPLE 13

Preparation of Compositions Containing D609/KC12 in the Steroid-Based Carrier Stearylamine For Therapeutic Test Purposes

The composition is prepared in a manner similar to that of Example 2 by mixing 2 grams of D609 and 2 grams of KC12 with 4 grams of stearylamine. Sterile, pyrogen-free water is added to make up a final volume of

40 ml of suspension. The suspension is placed in an external ice bath and sonified. The results achieved with this composition are similar to the results achieved with the composition of Example 2, with respect to skin irritation levels (Examples 4 and 5), and with respect to antiviral and antitumor activity (Example 6).

EXAMPLE 14

Preparation of Compositions Containing D609/KC12 in the Steroid-Based Carrier Chondrillasterol For Therapeutic Test Purposes

The composition is prepared in a manner similar to that of Example 2 by mixing 2 grams of D609 and 2 grams of KC12 with 4 grams of chondrillasterol. Sterile, pyrogen-free water is added to make up a final volume of 40 ml of suspension. The suspension is placed in an external ice bath and sonified. The results achieved with this composition are similar to the results achieved with the composition of Example 2, with respect to skin irritation levels (Examples 4 and 5), and with respect to antiviral and antitumor activity (Example 6).

EXAMPLE 15

Preparation of Compositions Containing D609/KC12 in the Steroid-Based Carrier α,β,γ Sitosterol For Therapeutic Test Purposes

The composition is prepared in a manner similar to that of Example 2 by mixing 2 grams of D609 and 2 grams of KC12 with 4 grams of α,β,γ sitosterol. Sterile, pyrogen-free water is added to make up a final volume of 40 ml of suspension. The suspension is placed in an external ice bath and sonified. The results achieved with this composition are similar to the results achieved with the composition of Example 2, with respect to skin irritation levels (Examples 4 and 5), and with respect to antiviral and antitumor activity (Example 6).

EXAMPLE 16

Preparation of Compositions Containing D609/KC12 in the Steroid-Based Carrier Cholestanol For Therapeutic Test Purposes

The composition is prepared in a manner similar to that of Example 2 by mixing 2 grams of D609 and 2 grams of KC12 with 4 grams of cholestanol. Sterile, pyrogen-free water is added to make up a final volume of 40 ml of suspension. The suspension is placed in an external ice bath and sonified. The results achieved with this composition are similar to the results achieved with the composition of Example 2, with respect to skin irritation levels (Examples 4 and 5), and with respect to antiviral and antitumor activity (Example 6).

EXAMPLE 17

Preparation of Compositions Containing D609/KC12 in the Steroid-Based Carrier Cholanic Acid For Therapeutic Test Purposes

The composition is prepared in a manner similar to that of Example 2 by mixing 2 grams of D609 and 2 grams of KC12 with 4 grams of cholanic acid. Sterile, pyrogen-free water is added to make up a final volume of 40 ml of suspension. The suspension is placed in an external ice bath and sonified. The results achieved with this composition are similar to the results achieved with the composition of Example 2, with respect to skin irritation levels (Examples 4 and 5), and with respect to antiviral and antitumor activity (Example 6).

WHAT IS CLAIMED IS:

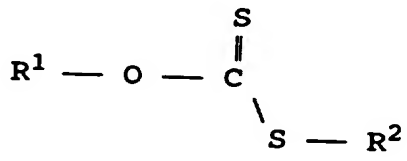
1. A pharmaceutical composition having antiviral or antitumor activity comprising an effective amount of (a) a xanthate compound and (b) an activity enhancing adjuvant, incorporated in a lipid-based or steroid-based carrier.

2. A composition of claim 1 wherein the composition is an antiviral agent.

3. A composition of claim 1 wherein the composition is an antitumor agent.

4. A pharmaceutical composition having antiviral or antitumor activity comprising:

(a) a xanthate compound of the formula (I):



(I)

wherein

R^1 represents norbornyl, tricyclodecyl, benzyl, straight or branched C_3-C_{20}-alkyl, C_3-C_{20}-cycloalkyl, furyl, pyridyl, quinuclidinyl; straight or branched C_3-C_{20}-alkyl substituted by hydroxy, C_1-C_4-alkoxy, or halogen; or C_3-C_{20}-cycloalkyl substituted by hydroxy, C_1-C_4-alkoxy, C_1-C_4-alkyl, or halogen; and

R^2 represents a monovalent or multivalent metal atom, straight or branched C_1-C_6-alkyl, straight or branched C_1-C_6-alkyl substituted by hydroxy, C_1-C_4-alkoxy, amino, C_1-C_4-alkylamino, $(C_1$-C_4-alkyl$)_2$-amino, $(C_1$-C_4-alkyl$)_3$-ammonium, or halogen; or 2,3-dihydroxypropyl or ω-hydroxy-$(C_1$-C_4-alkoxy)-methyl,

or a pharmaceutically acceptable salt thereof;

(b) an activity enhancing adjuvant comprising a compound having both a lipophilic group and a hydrophilic group, wherein the lipophilic group comprises an aliphatic group with six to eighteen carbon atoms, and the hydrophilic group comprises one or two carboxyl, sulphate, sulphonate, or phosphate groups, or a pharmaceutically-acceptable salt thereof; and

(c) a lipid-based or steroid-based carrier.

5. A composition of claim 4 wherein the xanthate is sodium or potassium benzylxanthate, cyclohexylxanthate, 1-adamantylxanthate, 8(9)-tricyclo[5.2.1.$0^{2.6}$]-decylxanthate, 2-endo or exo-bicyclo[2.2.$1^{1.4}$]-heptyl-xanthate, cyclododecylxanthate, n-dodecylxanthate, or 4-isobornyl-cyclohexylxanthate.

6. A composition of claim 4 wherein the adjuvant compound is an ionic compound having both lipophilic and hydrophilic groups, wherein the lipophilic group is a straight or branched aliphatic mono or dicarboxylic acid, or fluorinated derivative thereof, or an aliphatic mono or disulphate, mono or disulphonate, or mono or diphosphate, having 6 to 18 carbon atoms, or such a compound having 1 or 2 ether and/or amide groups, and wherein the hydrophilic group comprises 1 or 2 carboxyl and/or 1 or 2 sulphate, sulphonate, or phosphate groups, or pharmaceutically acceptable salts thereof.

7. A composition of claim 4 wherein the adjuvant compound is the sodium or potassium salt of decanoic acid, undecanoic acid, dodecanoic acid, deoxycholic acid, dodecyl sulfate, or dodecylphosphonic acid.

8. A composition of claim 4 wherein the weight ratio of xanthate to adjuvant is from 0.1 to 10 parts xanthate per one part adjuvant.

9. A composition of claim 4 wherein the weight ratio of xanthate to adjuvant is about 1:1.

10. A composition of claim 4 wherein the weight ratio of xanthate and adjuvant to carrier is one part xanthate and adjuvant to 0.25 to 4 parts carrier.

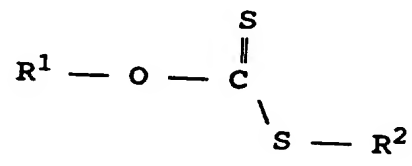
11. A composition of claim 4 wherein the carrier is cholesterol.

12. A composition of claim 4 wherein the xanthate and adjuvant are emulsified with said cholesterol form a micelle therewith or are encapsulated in a liposome made therefrom.

13. A composition of claim 4 wherein the active xanthate is sodium or potassium-8(9)-tricyclo[5.2.1.$0^{2.6}$]-decylxanthate, the activity increasing adjuvant is the sodium or potassium salt of dodecanoic acid, and the carrier is cholesterol.

14. A method of combating a virus or tumor comprising administering to a site of viral disease or to a tumor, an effective amount of a pharmaceutical composition comprising:

(a) a xanthate compound of the formula (I):



(I)

wherein

R^1 represents norbornyl, tricyclodecyl, benzyl, straight or branched C_3-C_{20}-alkyl, C_3-C_{20}-cycloalkyl, furyl, pyridyl, quinuclidinyl; straight or branched C_3-C_{20}-alkyl substituted by hydroxy, C_1-C_4-alkoxy, or halogen; or C_3-C_{20}-cycloalkyl substituted by hydroxy, C_1-C_4-alkoxy, C_1-C_4-alkyl, or halogen; and

R^2 represents a monovalent or multivalent metal atom, straight or branched C_1-C_6-alkyl, straight or branched C_1-C_6-alkyl substituted by hydroxy, C_1-C_4-alkoxy, amino, C_1-C_4-

alkylamino, $(C_1-C_4\text{-alkyl})_2$-amino, $(C_1-C_4\text{-alkyl})_3$-ammonium, or halogen; or 2,3-dihydroxypropyl or ω-hydroxy-$(C_1-C_4$-alkoxy)-methyl,

or a pharmaceutically acceptable salt thereof;

(b) an activity enhancing adjuvant comprising a compound having both a lipophilic group and a hydrophilic group, wherein the lipophilic group comprises an aliphatic group with six to eighteen carbon atoms, and the hydrophilic group comprises one or two carboxyl, sulphate, sulphonate, or phosphate groups, or a pharmaceutically-acceptable salt thereof; and

(c) a lipid-based or steroid-based carrier.

15. A method of claim 14 wherein the pharmaceutical composition comprises at least 2.5 wt% xanthate compound in a topical ointment.

16. A method of claim 14 wherein the pharmaceutical composition comprises at least 10 mg/ml xanthate in a solution for intravenous or subcutaneous injection.

17. A method of claim 14 wherein the xanthate is the sodium or potassium benzylxanthate, cyclohexylxanthate, 1-adamantylxanthate, 8(9)-tricyclo[5.2.1.$0^{2.6}$]-decylxanthate, 2-endo or exo-bicyclo[2.2.$1^{1.4}$]-heptyl-xanthate, cyclododecylxanthate, n-dodecylxanthate, or 4-isobornyl-cyclohexylxanthate.

18. A method of claim 14 wherein the adjuvant compound is an ionic compound having both lipophilic and hydrophilic groups, wherein the lipophilic group is a straight or branched aliphatic mono or dicarboxylic acid, or fluorinated derivative thereof, or an aliphatic mono or disulphate, mono or disulphonate, or mono or diphosphate, having 6 to 18 carbon atoms, or such a compound having 1 or 2 ether and/or amide groups, and wherein the hydrophilic group comprises 1 or 2 carboxyl

and/or 1 or 2 sulphate, sulphonate, or phosphate groups, or pharmaceutically acceptable salts thereof.

19. A method of claim 14 wherein the adjuvant compound is the sodium or potassium salt of decanoic acid, undecanoic acid, dodecanoic acid, deoxycholic acid, dodecyl sulfate, or dodecylphosphonic acid.

20. A method of claim 14 wherein the weight ratio of xanthate to adjuvant is 0.1 to 10 parts xanthate to one part adjuvant.

21. A method of claim 14 wherein the weight ratio of xanthate to adjuvant is one to one.

22. A method of claim 14 wherein the weight ratio of xanthate/adjuvant mixture to carrier is one part xanthate/adjuvant mixture to 0.25 to 4 parts carrier.

23. A method of claim 14 wherein the steroid is cholesterol.

24. A method of claim 14 wherein the xanthate and adjuvant are emulsified within said cholesterol or are encapsulated in a liposome made therefrom.

25. A method of claim 14 wherein the active xanthate is sodium or potassium-8(9)-tricyclo[5.2.1.$0^{2,6}$]-decylxanthate, the activity increasing adjuvant is the sodium or potassium salt of dodecanoic acid and the carrier is cholesterol.

FIG. 1

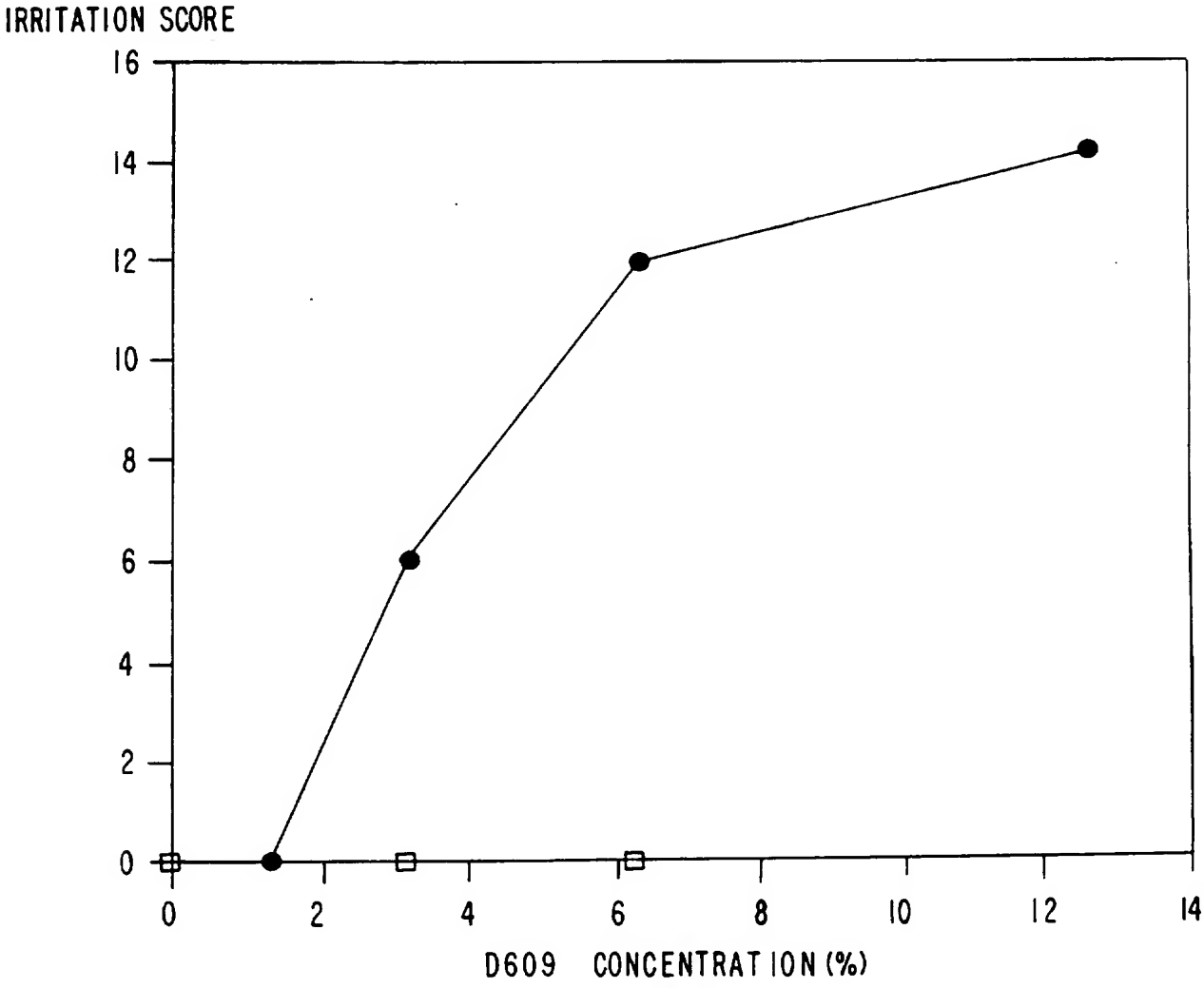
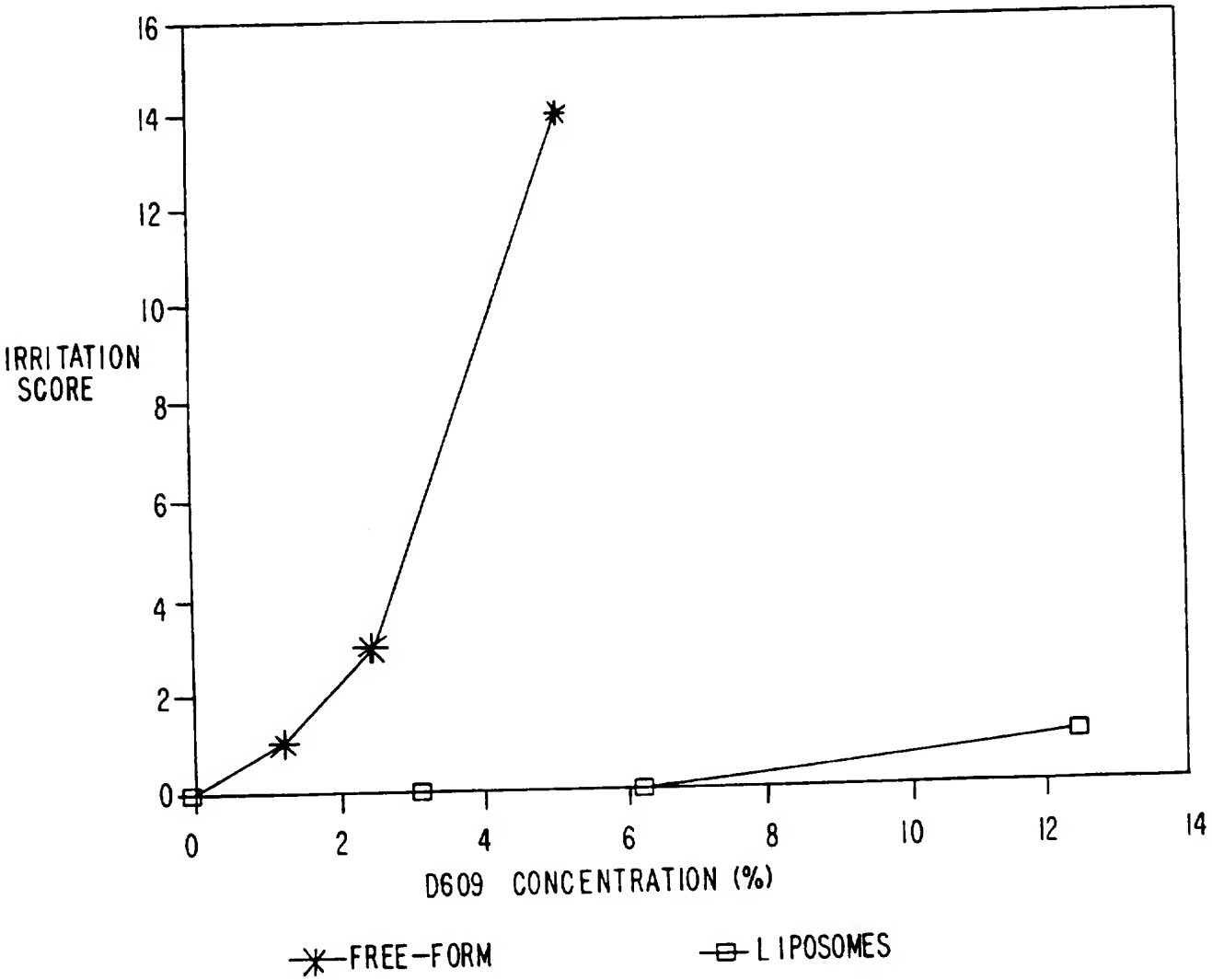

IRRITATION SCORE
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
D609 CONCENTRATION (%)

FIG. 2


IRRITATION SCORE
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
D609 CONCENTRATION (%)
FREE-FORM
LIPOSOMES

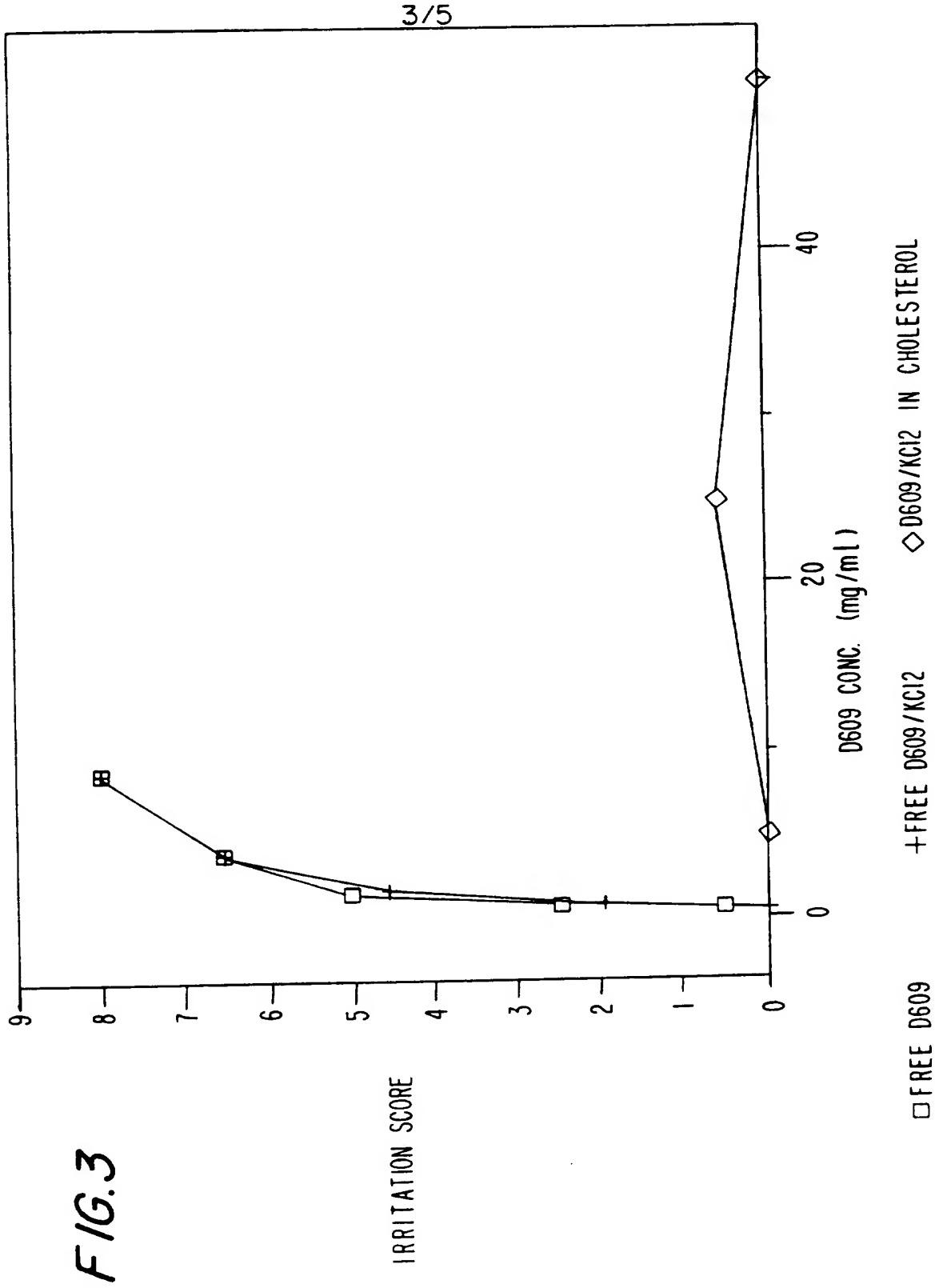

IRRITATION SCORE
0
1
2
3
4
5
6
7
8
9
D609 CONC. (mg/ml)
0
20
40
□FREE D609
+FREE D609/KC12
◇D609/KC12 IN CHOLESTEROL

FIG. 3

FIG.4

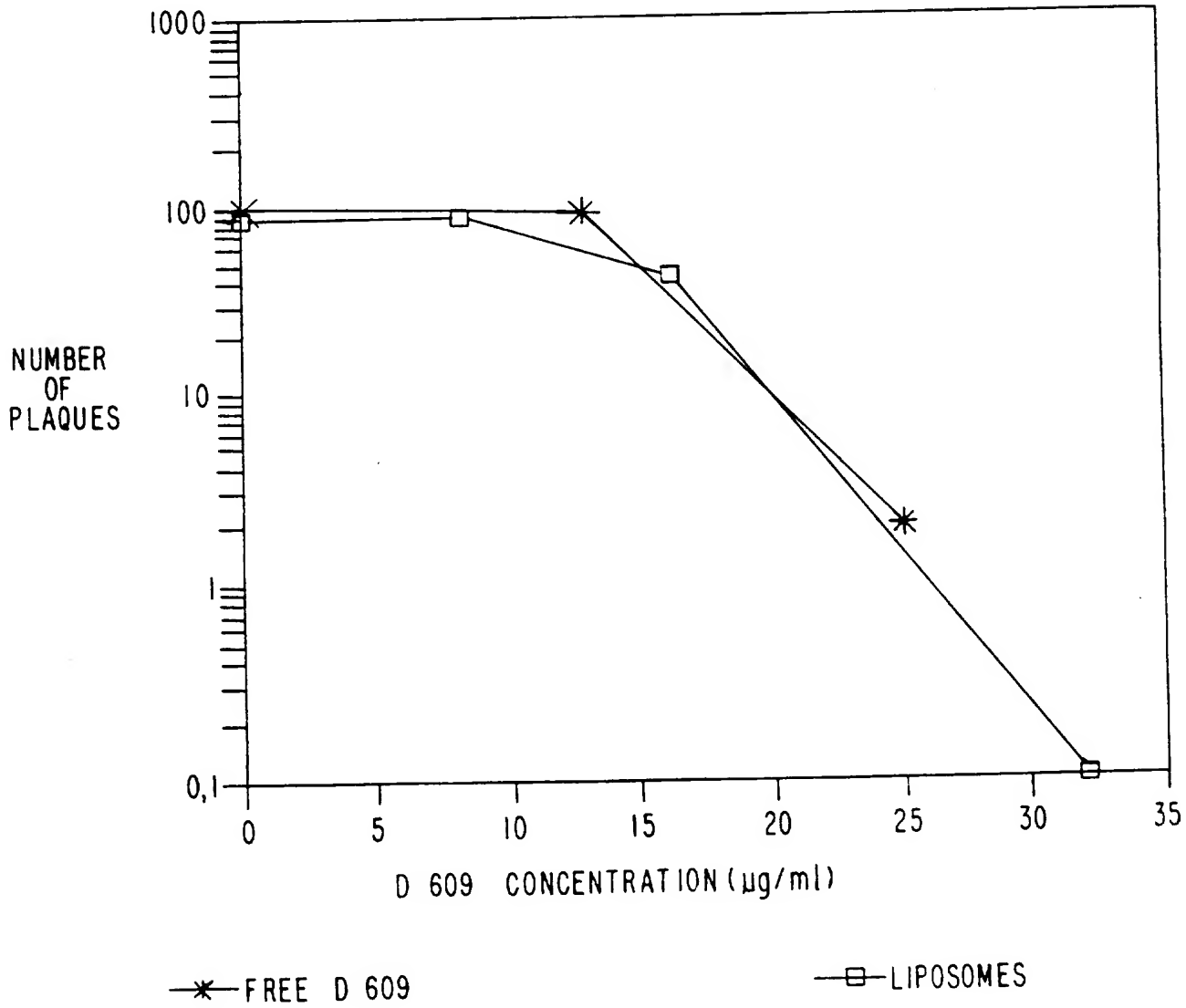
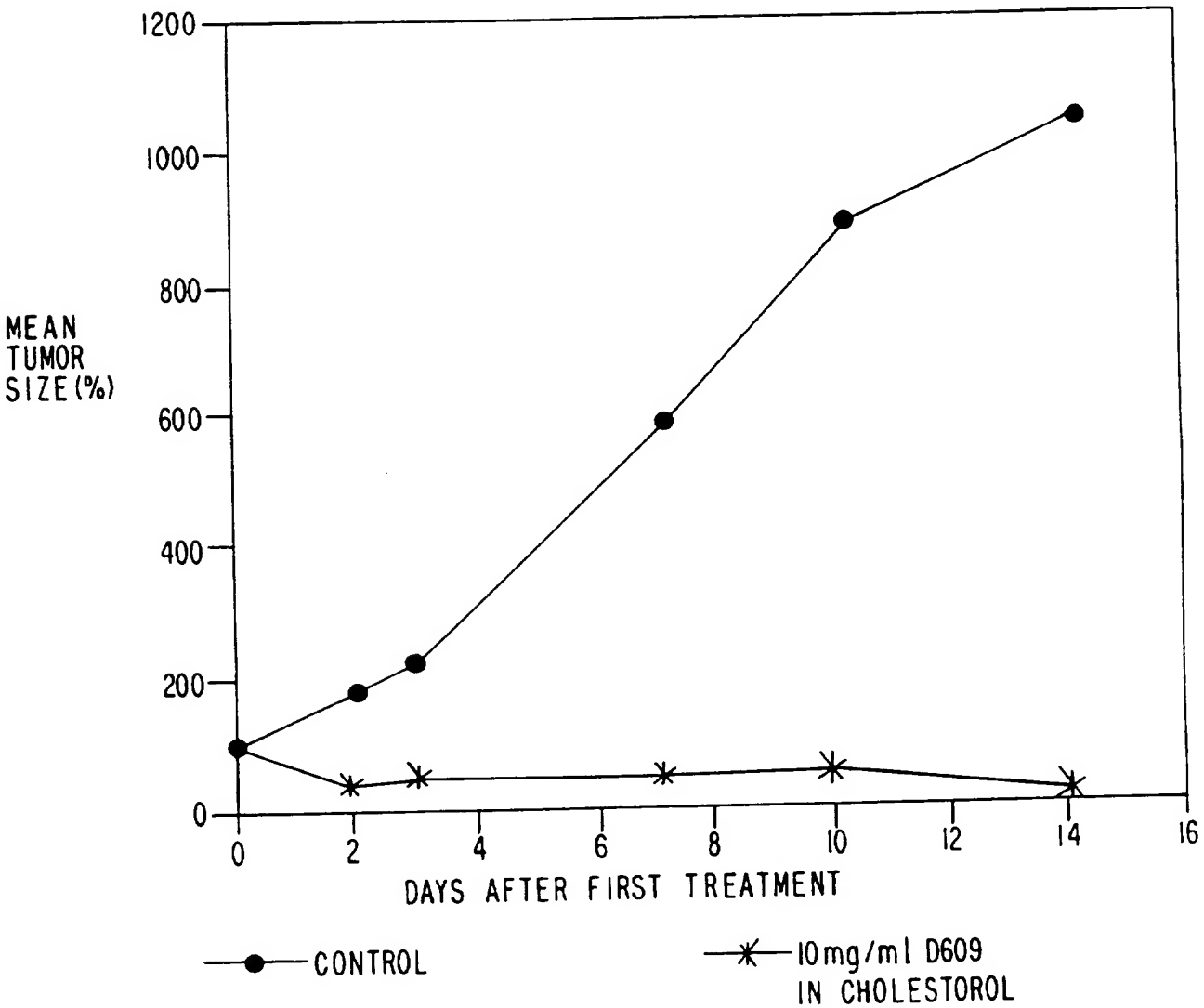

1000
100
10
1
0,1
NUMBER OF PLAQUES
0
5
10
15
20
25
30
35
D 609 CONCENTRATION (µg/ml)
FREE D 609
LIPOSOMES

FIG. 5


MEAN TUMOR SIZE (%)
1200
1000
800
600
400
200
0
0
2
4
6
8
10
12
14
16
DAYS AFTER FIRST TREATMENT
CONTROL
10mg/ml D609 IN CHOLESTOROL

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/14834

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/265; A61K 31/65
US CL :514/512; 171
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/512; 171

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,602,037 (SCHERM ET AL) 22 JULY 1986, see entire document.	1--25
Y	US, A, 4,851,435 (SAUER ET AL) 25 JULY 1989, see entire document.	1-25
Y	R. GENNARO et al, "REMINGTON'S PHARMECUTICAL SCIENCES", published 1985 by PHILIDELPHIA COLLEGE OF PHARMACY AND SCIENCE, (Philadelpha , PA) pages 1296-1298, see pages 1296-1298.	1-25

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

14 FEBRUARY 1996

Date of mailing of the international search report

04 MAR 1996

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
RUSSELL TRAVERS
Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/14834

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

I Claims 1-13 and 14-25 in part, drawn to an antiviral composition and methods for using this antiviral composition.
II Claims 1-13 and 14-25 in part, drawn to an antitumor composition and methods for using this antitumor composition.
The invention of group I describes an antiviral composition and antiviral theraputic methods, while group II describes antitumor compositions and antitumor theraputic methods. The two inventions do not share a common technical feature since group I is directed to antiviral therapy and group II is directed to antitumor therapy.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-13 , 14-25 in part

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.